# Effects of UVA (320–400 nm) on the Barrier Characteristics of the Skin

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The stratum corneum serves as the major barrier to the entrance of most molecules into the skin. In the studies presented here, the effects of UVA radiation (320–400 nm) on the barrier capacity of human stratum corneum were examined. Penetration of a homologous series of primary alcohols through unirradiated (control) and UVA-irradiated (test) human epidermis was determined in vitro. Permeability constants, $k_p$, were calculated. Mean ratios of permeability constants for UVA-irradiated and unirradiated epidermis (mean $k_p$ test)/(mean $k_p$ control) ranged from 2.3 to 3.0 for methanol and from 2.2 to 2.5 for ethanol. These mean ratios were determined using different pieces of epidermis from the same piece of skin for test and control samples. When $k_p$ control and $k_p$ test were determined on the same piece of epidermis on successive days, the ratios ($k_p$ test/$k_p$ control) were similar to the mean ratios determined on different pieces of epidermis. For other primary alcohols, propanol, butanol, hexanol, and heptanol, UVA radiation did not alter their permeability constants significantly. Partition coefficients, $K_m$, were determined for ethanol and heptanol using UVA-irradiated and unirradiated stratum corneum. For ethanol, irradiation resulted in a 1.5 to 2.6 times increase in $K_m$. For heptanol, irradiation caused no change in $K_m$. These results demonstrate that the barrier capacity of stratum corneum for small, polar, primary alcohols is diminished (permeability increases) and for higher molecular weight less polar alcohols, is unaffected by small doses of UVA radiation. This increased permeability of small polar alcohols through human skin may be due to enhanced partitioning into UVA-irradiated stratum corneum, which was not apparent for a higher molecular weight less polar alcohol. *J Invest Dermatol 96:758–762, 1991*

The rate-limiting step in the penetration into and through the skin for most substances is diffusion through the stratum corneum [1]. The corneocytes in the stratum corneum are composed mostly of filamentous proteins (keratins), probably small amounts of intracellular lipids and glycosaminoglycans [2], and water; there are larger amounts of intercellular lipids. There are sebaceous lipids on the surface. The chemical composition of the corneocyte membrane is not well known. Diffusion of molecules through this chemically and structurally complex tissue is difficult and specific molecular pathways are usually unknown, though there is considerable speculation concerning routes through the skin [3]. It is reasonable to expect that different types of molecules, e.g., polar and non-polar, will choose different pathways.

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Abbreviations:
GC: gas chromatograph
$K_m$: partition coefficient
$k_p$: permeability constant
TEWL: transepidermal water loss
UV: ultraviolet radiation
UVA: long-wave ultraviolet radiation (320–400 nm)
UVB: short-wave ultraviolet radiation (280–320 nm)

The stratum corneum is a barrier to the entrance not only of molecules but also of photons. Long-wave ultraviolet radiation, UVA (320–400 nm), penetrates further into the skin than does short-wave ultraviolet, UVB (280–320 nm). At 350 nm and 300 nm, the absorption coefficients for fair-skinned humans are 170 $cm^{-1}$ and 1700 $cm^{-1}$, respectively [4]. Jacques et al [5] demonstrated that UVA, but not UVB, altered the barrier capacity of human skin to water transport. These investigators demonstrated an in vitro twofold enhancement of water permeability after human skin was irradiated with as little as 10 J/$cm^2$ UVA. Their in vivo transepidermal water-loss (TEWL) studies in humans showed similar enhancement of water transport at UVA-irradiated sites. This in vivo effect was reversible and TEWL returned to normal levels within 4 h after UVA irradiation. These investigators also showed that irradiation of stratum corneum in vitro with 20 J/$cm^2$ UVA resulted in a 1.7 times increase in the tissue's water-holding capacity.

Bisset et al [6] have shown that chronic exposure of hairless mice to UVB radiation alone or a combination of UVA and UVB radiation permanently alters TEWL. UVA alone had no significant effect on TEWL. However, after several weeks of exposure to a combination of UVA (100 J/$cm^2$) and UVB (60 mJ/$cm^2$) three times a week, TEWL was increased fourfold. These doses are 4 times (for UVA) and 3 times (for UVB) the minimum doses required to elicit a minimal erythema in a fair-skinned human. This increase in TEWL persisted for several weeks after the irradiation treatments were stopped.

Because the barrier capacity of the skin to the transport of water is altered by UVA irradiation, it is logical to inquire if its barrier capacity to the transport of other molecules is altered. If so, exposure to UVA in amounts humans may be expected to receive from sun exposure in a temperate climate during summer months or from exposure to UVA in a tanning salon might possibly permit the

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entrance of larger amounts of some drugs, toxins, allergens, or carcinogens to which a person may be exposed. Benefits and/or risks might be estimated if it were known what classes of molecules penetrate irradiated skin more rapidly. One may first ask if the penetration rates of polar and non-polar molecules are accelerated equally by irradiation. There is an extensive literature on the penetration of the homologous series of primary alcohols [7–9]. This series of relatively small molecules range from very polar to very non-polar (strongly hydrophilic to strongly lipophilic). If it is found that UVA alters the barrier capacity for one class of molecules and not the other, one may speculate on which portion of the skin is likely to be altered. If the barrier capacity is altered, the flux will change.

According to an expanded Fick's Law, flux is directly proportional to the partition coefficient and the diffusion constant and inversely proportional to the thickness of the membrane [1]. If flux changes, it may be possible to determine experimentally which of these three variables is responsible for the change. Partition coefficient or diffusion constant, but not thickness, may be altered by irradiation of skin in vitro. Partition coefficients for the alcohols between stratum corneum and water can be measured experimentally but diffusion constant is difficult to measure accurately. We shall determine if partition coefficient is altered for those chemicals whose flux changes following UVA exposure.

In the following studies we have measured the effect of two doses of UVA on the flux of several primary alcohols when penetrating human epidermis in vitro from weak aqueous solutions. We also have determined the partition coefficient of two alcohols, one polar and one non-polar, between stratum corneum and water.

## MATERIALS AND METHODS

**Skin Preparation** Human abdominal skin obtained at autopsy was used in these studies. The epidermis was separated from full-thickness skin by immersing it in water at 60°C for 30 sec. The epidermis was then carefully removed with forceps [10]. Using this technique for separation, most basal cells remain attached to the dermis. It has been our experience that the flux of water through epidermis obtained by this technique closely approximates the flux of water through full-thickness skin. For each experiment, six 3.0-cm diameter pieces of epidermis were punched from the same abdominal specimen.

**Irradiation Procedure** The spectral irradiance of a 2.5-kW xenon arc lamp ("Solar Simulator," Oriel, Stratford, CT) with a liquid filter (40 g $CoSO_4$ and 40 g $CuSO_4$ per liter $H_2O$) is essentially limited to the 330–400-nm waveband with a peak at 372 nm. The intensity of UVA radiation was measured for the integrated 320–400-nm waveband with a cosine-corrected UV spectroradiometer system, Model IL 1700, using an IL SEE 400 detector (International Light, Inc., Danvers, MA) with a peak sensitivity at 360 nm and half-power points at 330 and 385 nm. The average intensity used in these experiments was 20 mW/cm². The field of irradiation was uniform to within ± 10% over the entire field of exposure.

Epidermal samples were irradiated either on day 1 or day 2. On day 1, the stratum corneum side of three test samples was irradiated with 20 or 80 J/cm² UVA; three control samples from the same piece of skin received no UVA. On day 2, the unirradiated samples from day 1 were irradiated with either 20 or 80 J/cm² UVA. The irradiated samples from day 1 were not irradiated on day 2. A dose of 20 J/cm² UVA is equivalent to the dose a person would receive in an hour of midday exposure to the sun in summertime at 39 to 40 degrees North latitude (the latitude of Rockville, MD).* Because for some alcohols there was no effect with 20 J/cm² UVA, we wished to determine if a higher dose could alter barrier capacity; we arbitrarily chose a fourfold increase in UVA dose, 80 J/cm². Because it has been shown that UVA altered the barrier capacity to water only when the stratum corneum was equilibrated with an environment greater than 62% relative humidity [5], we chose to equilibrate the epidermal specimens for 24 h prior to and during irradiation with an environment of 93% relative humidity in a quartz chamber containing a small vial of 1.5 M $K_2CO_3$ salt solution.

**Penetration Measurements** To measure the penetration of alcohols through human skin, irradiation and control samples from a given specimen were placed in glass diffusion chambers designed for measuring the flux from liquids [11]. The skin was placed in the chamber so that the stratum corneum faced the donor side. The donor chamber was filled with an aqueous alcohol solution, ranging in concentration from 0.004 M to 0.5 M. Where water solubility did not permit a concentration of 0.5 M, saturated solutions were used. Although the concentrations of alcohol donor solutions differ, calculation of the permeability constant, $k_p$, for each experiment normalizes all results for donor concentration. The receptor chamber was filled with an aqueous solution of sodium chloride (0.9%). To assure a well-mixed receptor solution, a Teflon-coated stirring bar, activated by an external magnetic stirrer, was used. All chambers were kept in a stirred bath at 31°C (the average temperature of the cutaneous surface in vivo).

On day 1 the amount of alcohol reaching the receptor was quantified by taking a 2-μl sample every 30 to 60 min for 6 to 8 h and injecting it into a Gas Chromatograph (Varian GC, Model 2440-10). For penetration measured after day 1, samples were taken every 30 to 60 min for 6 to 8 h per d for up to 3 d. The Varian GC was interfaced with an Apple II computer. Penetration is expressed as a permeability constant ($k_p$), the flux of the alcohol divided by the concentration of the alcohol in the original donor.

After day 1 measurements were completed, the donors and receptors of all chambers were washed 3 times with 0.9% NaCl solution, and the solution was kept in the chambers overnight. On day 2 all chambers were separated and the unirradiated control samples from day 1 were irradiated, after which their permeability to alcohol was again determined. Alcohol permeability for a piece of skin irradiated on day 2 is compared to the alcohol permeability of the same unirradiated piece from day 1 for determining the alcohol permeability on the same piece of skin before and after irradiation.

The epidermal samples irradiated on day 1 were not irradiated again on day 2, but were retested for alcohol permeability to determine if they were damaged by handling and standing overnight in contact with 0.9% NaCl solution. Some epidermal samples were damaged by chamber separation and/or exposure to saline solutions and alcohol solutions. The epidermal samples were visually inspected for obvious damage. Those samples, which did not show visual damage but showed very high flux on day 2, were considered damaged and not included in the data. If fewer than two irradiated and two unirradiated epidermal samples remained intact after the day 1 protocol and subsequent handling, the day 2 protocol was not performed. From the day 1 results mean ratios were determined (mean $k_p$ test/mean $k_p$ control). From the day 2 results, single $k_p$ ratios were determined (day 2 $k_p$ test/day 1 $k_p$ control).

**Partition Coefficient Determinations** Ethanol and heptanol partition coefficients ($K_m$) were determined for unirradiated and UVA-irradiated stratum corneum, using a modification of the procedure described by Scheuplein et al [12]. Human stratum corneum was obtained by floating epidermis on phosphate-buffered 1% trypsin solution for 2 h at room temperature (22°C). The epidermal cells were then removed from the stratum corneum by gently rubbing with a damp cotton swab [13]. The stratum corneum was allowed to equilibrate at 93% relative humidity for 24 h. After equilibration, one half of the stratum corneum was irradiated with UVA (20 or 80 J/cm²) in the same manner described earlier for epidermal samples. The remaining half of the stratum corneum served as unirradiated control. To determine dry weights, irradiated and control stratum corneum pieces were allowed to air dry over-

* Hourly UVA (320–400 nm) doses on a cloudless day, July 1, 1976, at ground level (39°–40° N), Rockville, Maryland (personal communication, Frederick Urbach).

**Table I.** Day 1 Results: Permeability Constants, $k_p$, of Primary Alcohols for Unirradiated (control) and UVA-Irradiated (test) Human Epidermis In Vitro

| | Permeability Constant ($cm \cdot h^{-1} \times 10^3$) (mean ± SD, n = 3) | | | |
|---|---|---|---|---|
| | Control Epidermis (unirradiated) | Test Epidermis (UVA-irradiated) | | |
| Alcohol | No UVA | 20 J/cm² | 80 J/cm² | p Value |
| Methanol | 0.84 ± 0.23 | 1.91 ± 0.33 | | 0.01 |
| | 0.34 ± 0.05 | 1.02 ± 0.05 | | 0.001 |
| | 1.27 ± 0.22 | 3.25 ± 1.21 | | 0.049 |
| Ethanol | 0.33 ± 0.03 | 0.77 ± 0.14 | | 0.01 |
| | 0.94 ± 0.14 | 2.07 ± 0.51 | | 0.021 |
| | 0.65 ± 0.06 | 1.62 ± 0.45 | | 0.022 |
| Propanol | 3.54 ± 2.93 | 3.57 ± 1.78 | | 0.991 |
| | 1.36 ± 0.42 | 2.00 ± 0.13[a] | | 0.140 |
| | 1.19 ± 0.28 | | 0.87 ± 0.21 | 0.184 |
| Butanol | 3.41 ± 0.59 | 3.15 ± 0.41 | | 0.573 |
| | 3.62 ± 0.53 | | 3.65 ± 1.05 | 0.970 |
| Hexanol | 24.0 ± 5.30 | 19.3 ± 2.48 | | 0.238 |
| | 25.3 ± 9.34 | | 15.3 ± 5.31 | 0.181 |
| Heptanol | 65.89 ± 5.75 | 58.00 ± 9.17 | | 0.275 |
| | 75.90 ± 43.2 | 68.93 ± 11.3 | | 0.800 |
| | 123.5 ± 7.21 | | 88.50 ± 12.3 | 0.013[b] |
| | 52.25 ± 6.99 | | 45.32 ± 8.64 | 0.337 |

[a] Mean ± SEM, n = 2.
[b] This result shows a significant decrease in $k_p$.

night and then were placed over Drierite (Fisher Scientific, Medford, MA) in a sealed container for 3 d.

The irradiated and control pieces of dry stratum corneum were each further divided into 3 pieces. Each piece was weighed and subsequently placed into a 500-μl Reacti-vial (Pierce Chemical Company, Rockford, IL) with 400 μl of aqueous alcohol solution of known concentration. The amount of alcohol in the original aqueous solution and in the solution after 4 d equilibration with stratum corneum was determined by gas chromatography. The amount of alcohol in the weighed pieces of stratum corneum was calculated from the difference between the amount of alcohol in the 400 μl of control solution and the amount in the solution after contact with stratum corneum. The concentration of alcohol in the stratum corneum (μl alcohol/mg dry stratum corneum) divided by the concentration of alcohol in the aqueous solution after contact with the stratum corneum (μl alcohol/μl solution) is the partition coefficient.

**Statistical Analysis** All linear regression slopes calculated from permeability data had correlation coefficients > 0.90. Differences in irradiated and control specimens were analyzed for significance by a determination of p value using the Student t test. For these studies a p value < 0.05 is considered significant.

## RESULTS

**Day 1 Results** Means of the permeability constants for the alcohols when penetrating unirradiated and UVA-irradiated human epidermis in vitro are shown in Table I. These day 1 results demonstrate significant increases in the permeability constants for methanol and ethanol through UVA-irradiated epidermis, as compared to unirradiated epidermis. These increases in permeability constants were observed for up to 4 d. For UVA-irradiated epidermis, there were no significant increases in permeability constants for propanol, butanol, hexanol, and heptanol. As is seen in Table I, permeability constants for a particular substance may vary greatly from one epidermal sample to the next. Therefore, it is advisable to determine the mean ratios of the permeability constants for irradiated (test) and unirradiated (control) samples for each piece of epidermis. The mean ratios for irradiated epidermis versus mean ratios for unirradiated epidermis (mean $k_p$ test/mean $k_p$ control) and the sum of the standard deviations of day 1 permeability constants were determined for each experiment (n = 3) and are shown in Fig 1. The mean ratios for methanol and ethanol ranged from 2.3 to 3.0 and 2.2 to 2.5, respectively. The other alcohols tested had mean ratios ranging from 0.7 to 1.5.

**Day 2 Results** The permeability constants for day 2 irradiated (test) samples were compared with the permeability constants for day 1 unirradiated (control) samples. Because the number of samples that seemed undamaged through the day 2 protocol were insufficient to determine mean ratios, permeability constant ratios of single pieces of epidermis were calculated (day 2 $k_p$ test/day 1 $k_p$ control). The day 2 single $k_p$ ratios, shown in Fig 2, are similar to the day 1 mean ratios. The ratios for methanol and ethanol ranged from 1.9 to 2.5 and 1.6 to 2.5, respectively. The other alcohols tested had ratios ranging from 0.5 to 1.3.

**Partition Coefficients, $K_m$** Means of ethanol and heptanol partition coefficients for UVA-irradiated and unirradiated human stratum corneum are shown in Table II. Ethanol partition coefficients for irradiated stratum corneum were significantly higher than those

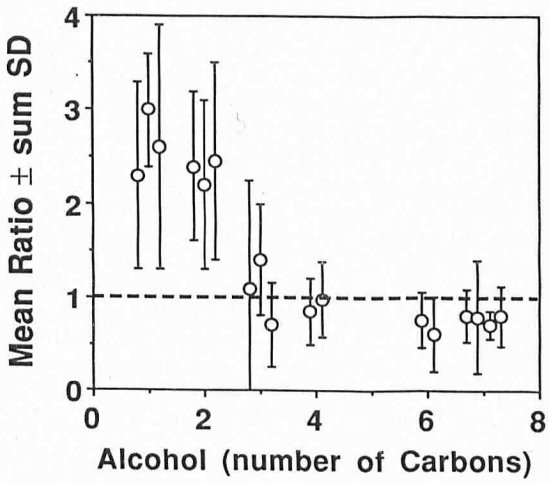


**Figure 1.** Day 1 mean ratios ± sum SD of permeability constants, $k_p$, for primary alcohols through human epidermis in vitro. Mean ratio = (mean $k_p$ test/mean $k_p$ control); n = 3.

**Table II.** Stratum Corneum/Water Partition Coefficients, $K_m$, for Ethanol and Heptanol Using UVA-Irradiated (test) and Unirradiated (control) Human Stratum Corneum (SC)

| | Partition Coefficient (mean ± SD, n = 3) | | | |
|---|---|---|---|---|
| | Control SC (unirradiated) | Test SC (UVA-irradiated) | | Mean Ratio, Test/Control |
| Alcohol | No UVA | 20 J/cm² | 80 J/cm² | |
| Ethanol[a] | 1.10 ± 0.4 | 2.90 ± 0.4 | | 2.64 |
| | 1.54 ± 0.4 | 2.37 ± 0.3 | | 1.54 |
| | 2.07 ± 0.5 | 3.86 ± 0.7 | | 1.86 |
| Heptanol[b] | 20.5 ± 7.6 | 16.0 ± 2.3 | | 0.78 |
| | 29.1 ± 6.8 | | 24.7 ± 7.9 | 0.85 |
| | 16.5 ± 7.5 | | 16.6 ± 4.4 | 1.01 |
| | 17.7 ± 7.1 | 11.0 ± 2.9 | | 0.62 |
| | 29.6 ± 9.2 | | 33.2 ± 7.8 | 1.12 |
| | 18.6 ± 2.8 | | 17.3 ± 3.4 | 0.93 |

[a] $p < 0.05$ for ethanol results.
[b] $p > 0.05$ for heptanol results.

for untreated stratum corneum ($p < 0.05$), when measured after 3 d of drying. UVA irradiation thus seems to induce changes in the stratum corneum that do affect the partition coefficient of ethanol. We do not know how long these changes last, but they last at least 3 d. There were no significant differences between the heptanol partition coefficients of unirradiated and irradiated stratum corneum.

## DISCUSSION

Our data indicate that exposure of the skin to low doses of UVA radiation results in an increased flux of methanol and ethanol when penetrating the epidermis from weak aqueous solutions. This increased permeability lasts for at least 3 d in vitro. No increase was seen for the higher molecular weight alcohols after exposures of 20 J/cm² UVA or when higher exposures of 80 J/cm² were used. Earlier studies in our laboratory have demonstrated increased TEWL through UVA-irradiated human skin in vivo.This increased TEWL lasted only a few hours [5].

Molecular pathways by which molecules diffuse through the skin are not well understood. It is reasonable to expect polar molecules to seek pathways presenting polar sites such as the protein filaments. Non-polar molecules may seek lipid pathways such as the lipids in the intercellular spaces [14]. Proteins absorb more ultraviolet radiation than do the lipids, and this may cause chemical changes in the proteins.

A type of chemical change that might occur is one that would expose more polar sites, such as $-COOH$ and $-NH_2$. If this occurs the stratum corneum may be expected to become a better "solvent" for water and polar molecules. This has been seen experimentally, e.g., greater water-holding capacity [5] and higher partition coefficient of ethanol.

The increase in partition coefficient is only slightly less than the increase in permeability constant. This would indicate that any alterations in the stratum corneum resulting from irradiation with UVA cause minimal or no change in diffusion coefficient. We would not expect UVA irradiation in vitro to change the thickness of the tissue.

Because the proteins absorb UVB, it is not clear to us why UVB irradiation appears not to alter the barrier characteristics for water [5]. Also, we might have expected that because UVA irradiation causes an increase in flux of methanol and ethanol, there would have been an increase in flux of propanol and butanol, which are relatively polar. We have not measured the partition coefficients for these alcohols.

Hydration of the stratum corneum increases the diffusivity of water in this tissue [15] and decreases its barrier capacity to many substances, both polar and non-polar [5,16], and UVA irradiation has been shown to increase the water-holding capacity of this tissue. If, however, this were the only mechanism whereby the fluxes of methanol and ethanol were increased, the fluxes of the other alcohols might have been expected to increase. This was not observed.

The stratum corneum protects the body against the loss of water and electrolytes and against the entrance of potentially toxic substances. Because of its location on the surface of the body, the skin receives greater exposure to radiant energy, UV and visible radiation, than does any other organ. Much of the radiation is absorbed, which likely causes chemical changes and can alter the skin's barrier characteristics, such as its ability to resist the loss of endogenous water and the entrance of some small, polar molecules. Any increases in TEWL following UVA irradiation are small and would not be expected to alter water balance significantly. We have not measured the effect of UVA on the penetration of drugs or toxic agents but would predict only a small increase, if any. This would be expected to have clinical significance only if a highly potent drug or a very toxic agent was penetrating.

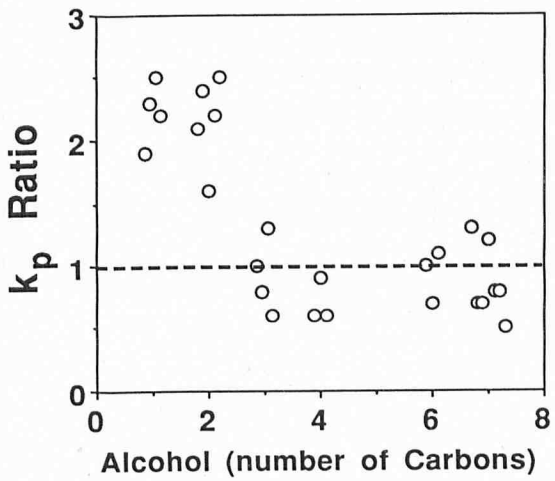


**Figure 2.** Day 2 ratios of single permeability constants, $k_p$, for primary alcohols through human epidermis in vitro. Ratio = (day 2 $k_p$ test/day 1 $k_p$ control).

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